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PRIMER NOTE

Ten microsatellite loci from *Zamia integrifolia* (Zamiaceae)

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Abstract

Ten microsatellite loci isolated from *Zamia integrifolia* are described. All 10 are polymorphic, with three to 10 alleles across 36 members of a single population from South Florida. Heterozygosities ranged from 0.139 to 0.889. Two loci depart significantly from Hardy–Weinberg equilibrium, and exhibit heterozygote deficiency. One locus pair exhibits significant linkage disequilibrium. The primers have also successfully amplified loci from *Zamia portoricensis* and *Zamia ambliphyllidia*. These loci will be utilized for population studies in the Caribbean *Zamia pumila* complex.

Keywords: coontie, cycad, microsatellite, population genetics, simple sequence repeat, *Zamia*

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The *Zamia pumila* (Zamiaceae) complex is a monophyletic and diploid ($2n = 16$) assemblage of cycad populations restricted to the West Indies and the southeastern United States, currently considered to comprise nine species (Stevenson 1987a,b; Sabato 1990; Géigel 2003). *Zamia integrifolia* is found from extreme southeastern Georgia southward through peninsular Florida (including the Florida Keys), sporadically in the Bahamas, and in coastal areas of north central Cuba, the Cayman Islands, and south central Puerto Rico (Stevenson 1991).

We isolated microsatellite loci from a population of *Z. integrifolia* located on the grounds of the Montgomery Botanical Center, Miami, Florida, using a modification of the enrichment method of simple sequence repeat (SSR) marker development of Edwards *et al*. (1996). Genomic DNA was restricted, ligated to adaptors and amplified with polymerase chain reaction (PCR). The products were hybridized twice with biotin-labelled synthetic SSRs and isolated using streptavidin-coated beads (Dynal) in conjunction with a Dynal Magnetic Particle Concentrator. The eluted fragments were size-separated using Sepharose CL-4B SizeSeptember 400 Spun Columns (Amersham Pharmacia Biotech), amplified and cloned (using phage and plasmid vectors with M13 priming sites), and the clones screened by sequencing. Reverse sequences were obtained for those containing a repeat, and primers were designed from the flanking regions of the consensus sequence using the Prime module of the Wisconsin Package version 10.2 for UNIX (Genetics Computer Group). If successful and polymorphic, a fluorescently labelled forward primer was obtained. The 10 primer pairs (Table 1) were tested across a sample from a wild population located on our location in Miami-Dade County, Florida. Differences in allele size were detected on an ABI 3730 Genetic Analyser (Applied Biosystems) using capillary gel electrophoresis with GENESCAN ROX-500 size standard (Applied Biosystems). PCR mix for all primers was 1× buffer (with 15 mM $MgCl_2$), 200 μM dNTPs, 250 nM each forward and reverse primer, 0.25 U *Taq* DNA polymerase (New England Biolabs), 10 ng genomic DNA template and nuclease-free distilled water to a total volume of 10 μL. We used the following PCR program on an ABI 9700 thermocycler (Applied Biosystems), adjusting annealing temperature and number of cycles as indicated (Table 1): 2 min at 94 °C, 35 or 38 cycles of (30 s at 94 °C, 1 min at 58–67 °C, 1 min at 72 °C), 10 min at 72 °C and 4 °C storage. Preliminary analysis of raw microsatellite data was performed using GENEMAPPER 3.5 (Applied Biosystems). Descriptive statistics (Table 1) were generated with GENALEX 6 (Peakall & Smouse 2006). Tests for null alleles were conducted with MICROCHECKER (Oosterhout *et al*. 2004). Tests for Hardy–Weinberg equilibrium (HWE), the exact test of probability (Haldane 1954; Weir 1990; Guo & Thompson 1992), and the *U*-test (Rousset & Raymond 1995) for heterozygote excess or deficiency, as well as linkage disequilibrium were run with GENEPOP 3.4 (Raymond & Rousset 1995).

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Table 1 Primer sequences and characteristics of 10 *Zamia integrifolia* microsatellite loci

Locus	GenBank Accession no.	Forward primer (5′–3′)	Reverse primer (5′–3′)	Repeat type	Dye (forward primer)	T_a (°C)	*n*	No. of alleles	Allele size range (bp)	Mean H_E	Mean H_O	f
Zam28	EF108366	CAGGACAAAAGCATATAGGC	TTGGTTTGGTAGGTGATAGG	$(GT)_{12}$	HEX	61	36	3	197–205	0.131	0.139	–0.062
Zam29	EF108367	GACTTCCTACCTAAAGATGCTA	TCCTGCTTACTTTAGATGCT	$(AGT)_{15}$	FAM	65	36	5	188–245	0.469	0.472	–0.007
Zam32	EF108368	CCAACAAGATAGCAAGGGA	AGCATGTGTAAACCTATAAAGGA	$(GT)_6(GA)_{14}$	HEX	60	36	4	145–151	0.602	0.556	0.078
Zam33	EF108369	CATGCTTGTTTGACTTGAACTA	TCCTTTCACACGTAATTATAAATCT	$(CA)_{13}$	FAM	58	36	6	187–203	0.738	0.389	0.473**
Zam34	EF108370	AGCATCAAGGGACTATCAAC	TCAATGCCTCTAATACAAAAAAAC	$(CTT)_9$	HEX	58	36	4	198–219	0.549	0.500	0.089
Zam35	EF108371	TGCATATACATATATACTCATGCAC	TCACTATGCACTCACATACATAA	$(TG)_{14}(TA)(TG)_{10}$	FAM	58	36	7	89–105	0.766	0.861	–0.124
Zam39	EF108372	TCACAAAATCTCAGCACCCC	TGAGTTAAACATGCGCCTCC	$(GA)_{14}$	FAM	60	36	5	166–184	0.718	0.639	0.110*
Zam40	EF108373	CTTGGCTACCTTCTCTCCTATC	TGTTTGGCTTCACCCTGTTC	$(AG)_{19}$	HEX	60	36	10	214–238	0.795	0.889	–0.118
Zam41	EF108374	GTAACAATCCCATTCCCCAAG	AGTGCCAAACCTCCAAGAAG	$(TG)_7G(GA)_{14}$	FAM	67	36	5	225–235	0.656	0.611	0.068
Zam45	EF108375	CCTTAAAGAAGCTCGTAACTCC	TTCCCGAGATTATCCCTAGC	$(GA)_9$	FAM	55	36	3	141–145	0.610	0.667	–0.093

n, sample size; H_E, expected heterozygosity; H_O, observed heterozygosity; f, estimate of fixation index; T_a, annealing temperature.
†35 amplification cycles in PCR.
‡38 amplification cycles in PCR.
*Departs significantly from HWE at $P < 0.05$, **< 0.001.

None of the loci was monomorphic across the test population, and all 36 individuals in the sample had unique multilocus genotypes. Two loci depart significantly from HWE, Zam33 at $P < 0.05$, and Zam39 at $P < 0.001$. Both of these loci also exhibit significant heterozygote deficiency ($P < 0.001$ and $P < 0.05$, respectively). One locus (Zam33) showed evidence for a null allele. Only one pairwise combination of loci (Zam28 and Zam33) exhibited significant linkage disequilibrium at $P < 0.01$.

The primers also successfully amplified loci from *Zamia amblyphyllidia* D.W. Stev. and *Zamia portoricencis* Urb. Though some of our previously isolated primers worked on *Zamia* species from Central and South America (Meerow *et al*. in press), we have not yet experimented with any of these 10 on those templates.

We plan to use these SSR loci along with seven others previously isolated (Meerow *et al*. in press) to investigate patterns of genetic variation in the complex. In the last 200 years, the ecosystems of the West Indies and Florida have been heavily transformed by urban and agricultural development (Myers & Ewel 1990; Adams 1997; Myers *et al*. 2000), with habitat fragmentation concentrated in the lower elevations of the entire region where *Zamia* is most often found (Whitelock 2002). We thus anticipate that an understanding of the genetic structure of the populations of this complex will help to delineate future conservation strategies for the group.

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